Regulation of succinate-fuelled mitochondrial respiration in liver and skeletal muscle of hibernating thirteen-lined ground squirrels.

Jason C L Brown
Dillon J Chung
Alex N Cooper
James F Staples

Follow this and additional works at: http://ir.lib.uwo.ca/biologypub

Part of the Biology Commons

Citation of this paper:
Brown, Jason C L; Chung, Dillon J; Cooper, Alex N; and Staples, James F, "Regulation of succinate-fuelled mitochondrial respiration in liver and skeletal muscle of hibernating thirteen-lined ground squirrels." (2013). Biology Publications. Paper 60.
http://ir.lib.uwo.ca/biologypub/60
RESEARCH ARTICLE

Regulation of succinate-fuelled mitochondrial respiration in liver and skeletal muscle of hibernating thirteen-lined ground squirrels

Jason C. L. Brown*, Dillon J. Chung, Alex N. Cooper and James F. Staples

Department of Biology, University of Western Ontario, London, ON, Canada, N6A 5B7

*Author for correspondence (jbrow63@alumni.uwo.ca)

SUMMARY

Hibernating ground squirrels (*Ictidomys tridecemlineatus*) alternate between two distinct metabolic states throughout winter: torpor, during which metabolic rate (MR) and body temperature (\(T_b\)) are considerably suppressed, and interbout euthermia (IBE), during which MR and \(T_b\) briefly return to euthermic levels. Previous studies showed suppression of succinate-fuelled respiration during torpor in liver and skeletal muscle mitochondria; however, these studies used only a single, saturating succinate concentration. Therefore, they could not address whether mitochondrial metabolic suppression occurs under physiological substrate concentrations or whether differences in the kinetics of mitochondrial responses to changing substrate concentration might also contribute to mitochondrial metabolic regulation during torpor. The present study confirmed that succinate oxidation is reduced during torpor in liver and skeletal muscle at 37 and 10°C over a 100-fold range of succinate concentrations. At 37°C, this suppression resulted from inhibition of succinate dehydrogenase (SDH), which had a greater affinity for oxaloacetate (an SDH inhibitor) during torpor. At 10°C, SDH was not inhibited, suggesting that SDH inhibition initiates but does not maintain mitochondrial suppression during torpor. Moreover, in both liver and skeletal muscle, mitochondria from torpid animals maintained relatively higher respiration rates at low succinate concentrations, which reduces the extent of energy savings that can be achieved during torpor, but may also maintain mitochondrial oxidative capacity above some lower critical threshold, thereby preventing cellular and/or mitochondrial injury during torpor and facilitating rapid recruitment of oxidative capacity during arousal.

Key words: enzyme kinetics, oxaloacetate, succinate dehydrogenase, succinate oxidation, torpor.

Received 27 July 2012; Accepted 13 January 2013

INTRODUCTION

Hibernating thirteen-lined ground squirrels (*Ictidomys tridecemlineatus* Mitchell), like other small mammalian hibernators, alternate between two distinct metabolic states throughout winter: torpor and interbout euthermia (IBE) (Young, 1990; Grahn et al., 1994; Brown et al., 2012). During torpor, metabolic rate (MR) is less than 5% of basal metabolic rate and core body temperature (\(T_b\)) is maintained at near-ambient levels (Geiser, 2004; Brown et al., 2012). Torpor bouts are spontaneously interrupted every 7–12 days by periods of IBE, during which \(T_b\) returns to ~37°C and MR is maintained at typical resting levels (10- to 20-fold higher than during torpor) for up to 9h. Transitions between torpor and IBE occur very rapidly: arousal from torpor to IBE takes only a few hours, and entrance into torpor from IBE occurs within a single day.

A tremendous amount of research has been devoted to elucidating the mechanisms by which hibernating animals can rapidly, reversibly and repeatedly suppress their metabolism to such a considerable extent. In particular, much attention has been paid to the role of mitochondrial oxidative phosphorylation, as this process is responsible for up to 90% of whole-animal oxygen consumption in mammals (Rolfé and Brown, 1997). A large number of studies, using succinate as a respiratory substrate, have shown that mitochondrial state 3 (phosphorylating) respiration rate is reduced up to 83 and 32% during torpor compared with IBE in liver (Gehnrich and Aprille, 1988; Muleme et al., 2006; Gerson et al., 2008; Armstrong and Staples, 2010; Chung et al., 2011; Brown et al., 2012) and skeletal muscle (Brown et al., 2012), respectively, when measured *in vitro* at 37°C. When measured at low, torpid-like temperatures (i.e. 4–10°C), lower respiration rates during torpor have been observed in some (Pehowich and Wang, 1984) but not all (Muleme et al., 2006; Brown et al., 2012) studies. Suppression of mitochondrial state 3 respiration during torpor has also been observed with several NADH-linked substrates, including pyruvate, glutamate and \(\beta\)-hydroxybutyrate, at least in liver (Fedotcheva et al., 1985; Gehnrich and Aprille, 1988; Brustovetsky et al., 1990; Muleme et al., 2006). On this basis, it has been proposed that active suppression of mitochondrial oxidative capacity occurs during torpor and may contribute to the reduction of whole-animal metabolism that characterizes mammalian torpor.

All previous studies of mitochondrial metabolism during hibernation used only a single, saturating substrate concentration in order to assess differences in maximal oxidative capacity between metabolic states. There are two problems with this approach. First, physiological substrate concentrations are probably less than saturating, so studies using only saturating substrate concentrations cannot address whether mitochondrial suppression may occur under *in vivo* conditions. Second, at the enzyme level, it has been proposed that changes to substrate affinity may play a greater role in regulating enzyme activity at *in vivo* substrate concentrations than changes in maximal enzyme activity (Somero, 1969). If the same is true at the mitochondrial level, then examining only maximal mitochondrial respiration rates may not allow for a complete
understanding of the regulation of mitochondrial metabolism during torpor. In particular, alterations in the kinetics of mitochondrial responses to changes in substrate concentration could either dampen or further enhance the effects of reduced oxidative capacity during torpor. Therefore, the first objective of the present study was to measure succinate-fuelled respiration at 37 and 10°C in liver and skeletal muscle mitochondria from torpid and IBE hibernators over a wide range of succinate concentrations (from 0.1 to 10 mmol l⁻¹).

A second objective of the present study was to further examine the role of succinate dehydrogenase (SDH) inhibition in the suppression of succinate-fuelled metabolism during torpor. Maximal SDH activity is significantly lower during torpor compared with IBE in liver (Gehnrich and Aprille, 1988; Armstrong and Staples, 2010; Chung et al., 2011), but, to our knowledge, no studies have examined SDH activity in skeletal muscle in any hibernating species. In addition, similar to the studies of mitochondrial respiration, SDH activity has always been measured at 37°C using only a single, saturating succinate concentration in order to obtain maximal rates; that is, no studies have examined whether SDH activity is inhibited at torpid-like temperatures, or whether there are changes in SDH kinetics during torpor. Therefore, we measured SDH activity in both liver and skeletal muscle at 37 and 10°C over the same range of succinate concentrations used to assess mitochondrial respiration rates. In addition, we examined whether respiration rates of intact mitochondria correlated with SDH activity measured in the same mitochondria at all succinate concentrations and both assay temperatures.

One of the most potent and well-characterized inhibitors of SDH is oxaloacetate (OAA) (Wojtczak et al., 1969; Zeylemaker et al., 1969), and a few studies of hibernators have suggested that OAA inhibits SDH activity during torpor (Fedotcheva et al., 1985; Armstrong and Staples, 2010; but see Brustovetsky et al., 1989). Armstrong and Staples (Armstrong and Staples, 2010) found no significant increase in liver OAA concentration during torpor in thirteen-lined ground squirrels. While this does not preclude the possibility that intramitochondrial OAA levels may increase during torpor, it might also suggest that SDH inhibition during torpor results from increased affinity of SDH for OAA. Therefore, the final objective of the present study was to examine the effects of OAA on liver and skeletal muscle SDH kinetics during torpor and IBE at both 37 and 10°C.

**MATERIALS AND METHODS**

**Animals**

This project conformed to the guidelines of the Canadian Council on Animal Care and was approved by the local Animal Use Subcommittee (protocol 2008-055-06). Thirteen-lined ground squirrels were live-trapped in late May in Carman, MB, Canada (49°30’N, 98°01’W). Additionally, some animals were born in captivity to wild-caught pregnant females. Both male and female individuals were used. Animals were housed individually in plastic cages (26.7×48.3×20.3 cm) and provided with corn-cob bedding, EnviroDri shredded paper bedding (used for nest building), a transparent red polycarbonate tube (for enrichment, bedding, EnviroDri shredded paper bedding (used for nest transport chain and prevent reverse electron flow. Succinate was added to inhibit complex I of the mitochondrial electron transport chain and prevent reverse electron flow. Succinate-fuelled respiration was measured by suspending liver (~30 μg protein) or skeletal muscle (~30 μg protein) mitochondria in 2.5 ml of MiR05 assay buffer (Gnaiger and Kuznetsov, 2002) equilibrated to either 37 or 10°C. We chose to use 10°C, our temperature slightly above torpid T₀ (~5°C), because, in our experience, it is the lowest in vitro temperature that yields reliable respiration measurements. Rotenone (2 μg ml⁻¹, dissolved in ethanol) was added to inhibit complex I of the mitochondrial electron transport chain and prevent reverse electron flow. Succinate (0.1–10 mmol l⁻¹) was added to stimulate state 2 respiration, and, subsequently, ADP (0.2 mmol l⁻¹) was added to stimulate state 3 respiration. Once a steady-state rate had been achieved, oligomycin (2 μg ml⁻¹, dissolved in ethanol; inhibitor of F₁F₀-ATPase) was added to approximate state 4 conditions.

**Succinate dehydrogenase activity and oxaloacetate affinity**

For measurements of SDH activity, mitochondria (sonicated on ice for 2×10 s with 30 s between; ~10 μg protein liver and ~3 μg protein skeletal muscle; or buffer alone for blanks) were added to 200 μl of SDH buffer (55 mmol l⁻¹ KH₂PO₄, 25 mmol l⁻¹ sucrose and 0.11% w/v/iodonitrotetrazolium chloride, pH 7.4 at respective assay temperature, either 37 or 10°C). Subsequently, succinate (0.05–10 mmol l⁻¹) was added to stimulate SDH activity. Absorbance (500 nm) was measured every 15 s for 10 min using a spectrophotometer (SpectraMax M2e, Molecular Devices, Sunnyvale, CA, USA), and initial linear rates were used to calculate kinetic parameters.

In July, radiotelemeters (TA-F10; Data Sciences International, St Paul, MN, USA) were implanted into the intraperitoneal space under isofluorane anaesthesia to allow for T₀ monitoring (Muleme et al., 2006). Ketoprofen (Aranifen; Merial Canada, Baie d’Urfé, QC, Canada; 10 mg ml⁻¹, 0.1 ml 100 g⁻¹) was provided as an analgesic prior, and for 3 days following, surgery. In October, animals began to hibernate and were moved to an environmental chamber maintained at 4±2°C on a 2 h:22 h light:dark photoperiod [lights on at 08:00 h Eastern Standard Time (EST)]. After 1 week of uninterrupted torpor, food (but not water) was withheld. All animals were sampled throughout winter (from December to March) at the same time of day (08:00–10:00 h EST). Torpid animals were sampled when T₀ was at or below 5°C for at least 3 days and were euthanized via cervical dislocation. IBE animals were sampled following spontaneous arousal from torpor when T₀ was at or above 36°C for at least 1 h and were euthanized via anaesthetic overdose (Euthanyl, Bimeda-MTC, Cambridge, ON, Canada; 270 mg ml⁻¹, 0.2 ml 100 g⁻¹). These different euthanasia methods are required by our animal care protocols, but Euthanyl has no effects on mitochondrial metabolism (Takagi et al., 1997).
occurs via competitive inhibition, as it occurs even at saturating succinate levels, so we chose to examine OAA inhibition of SDH by pre-incubating the enzyme with OAA in the absence of succinate. Mitochondria (concentrations as above) were added to 200 μl of SDH buffer and pre-incubated for 5 min with 5 μmol l⁻¹ OAA. This concentration of OAA was selected because it approximates the difference in tissue OAA concentration observed between torpor and IBE (Armstrong and Staples, 2010). Following pre-incubation, succinate was added to stimulate SDH activity and absorbance was measured as described above.

Data analysis
Values presented are means ± s.e.m., except where N=2, in which cases the range is shown. To examine differences in mitochondrial respiration rate and SDH activity between metabolic states over the range of succinate concentrations, mitochondrial respiration and enzyme activity data for each tissue and assay temperature were analyzed separately using a linear mixed model analysis with repeated measures (succinate concentration). To examine differences in enzyme kinetics between metabolic states, maximal enzyme activity (Vₘₐₓ) and apparent Michaelis–Menten constant (AppKₘ) were estimated using SigmaPlot 2001 (SPSS, Chicago, IL, USA) by fitting the mitochondrial respiration or SDH activity data to the Michaelis–Menten equation (single rectangular hyperbola). Inhibition constants (Kᵢ and Kᵢ') for OAA were determined by calculating Vₘₐₓ and AppKₘ in the absence and presence of OAA and solving the extended Michaelis–Menten equation for these parameters. Subsequently, kinetic parameters for each tissue were analyzed separately using a linear mixed model with repeated measures (temperature). In all analyses, non-significant interactions were removed from the model. All statistical analyses were conducted using SPSS version 19.

RESULTS Respiration rate is lower in mitochondria from torpid animals regardless of succinate concentration or assay temperature
When measured at 37°C, state 3 respiration rate of both liver (Fig. 1A) and skeletal muscle (Fig. 1B) mitochondria was significantly lower in torpid animals, regardless of succinate concentration used, with the exception of 0.1 mmol l⁻¹ succinate in liver. With saturating (10 mmol l⁻¹) succinate levels, the extent of suppression (52 and 33% in liver and skeletal muscle, respectively, compared with IBE) was comparable to previous studies (Gehnrich and Aprille, 1988; Muleme et al., 2006; Gerson et al., 2008; Armstrong and Staples, 2010; Chung et al., 2011; Brown et al., 2012), but the extent of suppression tended to decline with succinate concentration. For example, with 1 mmol l⁻¹ succinate, the extent of suppression was 42% in liver and 12.5% in skeletal muscle. Similar results were obtained when mitochondrial respiration rate was measured at 10°C. In liver (Fig. 1C), state 3 respiration rate was significantly lower (50–60%) in torpid animals down to 1 mmol l⁻¹ succinate, while at lower succinate levels, no difference was observed. In skeletal muscle (Fig. 1D), state 3 respiration was suppressed at all succinate concentrations, but the extent of suppression was greater at saturating levels (e.g. 51.5% at 10 mmol l⁻¹) compared with sub-saturating levels (e.g. 39% at 1 mmol l⁻¹).

Succinate dehydrogenase activity is lower in torpor at 37°C – but not 10°C – regardless of succinate concentration
We measured SDH activity at the same succinate concentrations and assay temperatures used to measure mitochondrial respiration. In order to better resolve SDH kinetics, we additionally measured SDH activity with 0.25 mmol l⁻¹ succinate at 37°C and 0.05 mmol l⁻¹ succinate at 10°C. At 37°C, SDH activity was significantly lower
in torpid animals compared with IBE in both liver (Fig. 2A) and skeletal muscle (Fig. 2B) regardless of succinate concentration, again with the exception of liver at 0.1 mmol l−1 succinate. As with mitochondrial respiration, the extent of inhibition of SDH tended to be higher at saturating succinate levels. In liver, SDH was 38% lower at 10 mmol l−1 succinate but only 30% lower at 1 mmol l−1, and, in skeletal muscle, SDH was 24% lower at 10 mmol l−1 succinate but only 18% lower at 1 mmol l−1. At 10°C, however, no difference in SDH activity between metabolic states was observed in either liver (Fig. 2C) or skeletal muscle (Fig. 2D) at any succinate concentration.

The extent of SDH inhibition during torpor correlated well with differences in the extent of suppression of mitochondrial succinate oxidation both between and within tissues when measured at 37°C. The extent of both SDH inhibition and mitochondrial succinate oxidation suppression were higher in liver compared with skeletal muscle (compare data in Figs 1 and 2). Moreover, within both liver and skeletal muscle, there was a strong correlation between SDH activity and mitochondrial succinate oxidation rate at all succinate concentrations where suppression of mitochondrial respiration was observed, with the notable exception of 0.5 and 0.1 mmol l−1 in skeletal muscle (Fig. 3); however, the strength of the correlation tended to decline at lower succinate levels in both tissues.

**Kinetics of mitochondrial respiration and SDH are altered during torpor, especially in liver**

The kinetics of both mitochondrial respiration (Fig. 4A) and SDH (Fig. 4B) were assessed by fitting data from Figs 1 and 2 to the Michaelis–Menten equation. This equation generally fitted the data quite well (r² > 0.9), except for respiration data measured at 10°C (r² = 0.49–0.85 depending on tissue and metabolic state). While this may reduce the accuracy of our estimates of AppKₘ values at 10°C for the mitochondrial respiration data, we believe that these values reflect the clear trends in the data. In both tissues and at both assay temperatures, the extent of decline in mitochondrial respiration rate at lower succinate concentrations was reduced in torpor. For example, for liver mitochondrial respiration measured at 37°C, the respiration rate measured using 1 mmol l−1 succinate was 48% lower compared with that measured using 10 mmol l−1 succinate for IBE mitochondria; however, the reduction was only 38% for torpid mitochondria over the same change in concentration. Similar findings were observed in skeletal muscle at 37°C, and both tissues at 10°C. Therefore, for liver mitochondrial respiration, we observed that AppKₘ was significantly lower in torpor than IBE at both assay temperatures. For skeletal muscle respiration, similar trends were seen, especially at 37°C, but they did not reach statistical significance.

In both liver and skeletal muscle, AppKₘ values were considerably (~5-fold) lower for SDH than mitochondrial respiration. In liver, similar to mitochondrial respiration, AppKₘ was significantly lower in torpor than IBE at both 37 and 10°C. In skeletal muscle, by contrast, as with mitochondrial respiration, AppKₘ values showed no significant difference between metabolic states for either mitochondrial respiration or SDH activity, though they tended to be lower in torpor at 37°C.

**SDH from torpid animals has a greater apparent affinity for oxaloacetate**

We also measured SDH kinetics following a 5-min pre-incubation with OAA in both liver (Fig. 5A) and skeletal muscle (Fig. 5B). Pre-incubation with OAA in the absence of succinate allowed OAA to act as a mixed inhibitor of SDH (Mandrik et al., 1983). Under these conditions, OAA can bind to SDH when succinate is not bound and also when succinate is already bound, although with different affinities, termed Kᵢ and Kᵢ′, respectively. We observed that OAA had a much lesser apparent affinity for SDH when succinate was previously bound than when it was not already bound (Kᵢ′ was 15- to 30-fold higher than Kᵢ), and that Kᵢ′ did not differ between
metabolic states in either tissue (data not shown). However, $K_i$ was significantly lower in torpor in both liver and skeletal muscle at 37 and 10°C (Fig. 5C), suggesting that the affinity of SDH for OAA is greater in torpor, regardless of assay temperature or tissue.

**Discussion**

In torpor, mitochondria exhibit reduced oxidative capacity over a range of succinate concentrations but are less responsive to changes in substrate concentration

The present study demonstrates that suppression of succinate-fuelled respiration occurs with both saturating and sub-saturating succinate concentrations. This observation is important because sub-saturating succinate concentrations probably better reflect physiological conditions. While, to our knowledge, there are no studies of intramitochondrial substrate levels during in vivo metabolism, reports of tissue succinate concentrations range from <0.1 mmol l$^{-1}$ in mouse liver and skeletal muscle (Lin et al., 2011) to <0.5 mmol l$^{-1}$ in rabbit heart (Lewandowski et al., 1996) to ~1 mmol l$^{-1}$ in liver from rats (Busch and Potter, 1952) and hibernating ground squirrels (Serkova et al., 2007). Bollard et al. (Bollard et al., 2003) suggest that succinate is largely a mitochondrial metabolite, being more readily detected in mitochondrial extracts than whole tissue extracts using NMR-based techniques; therefore concentrations reported for whole tissue probably underestimate intramitochondrial levels. Notwithstanding, we are confident that the range of succinate concentrations used in this study spans the physiological range. Thus the present study demonstrates that mitochondrial oxidative capacity is probably reduced during torpor in vivo and can play an important role in reducing MR.

The present study also demonstrates that mitochondria are less responsive to changes in substrate concentration in torpor than in IBE. In liver, this fact is reflected by the lower App$K_m$ value for succinate-fuelled respiration in torpor, and a similar trend was observed in skeletal muscle, at least at 37°C. This lower sensitivity to changing substrate levels in torpor means that current estimates of energy savings achieved through mitochondrial suppression are probably overestimates, because they are based on measurements made using saturating succinate concentrations where the difference in oxygen consumption rates between IBE and torpor is maximal. At sub-saturating substrate concentrations, probably more representative of physiological conditions, the difference between IBE and torpor is smaller. Therefore, while hibernator mitochondria are clearly modified to reduce their capacity to oxidize substrates during torpor, they appear to be simultaneously modified to ensure that mitochondrial respiration does not decline to very low levels during torpor but rather remains above some critical threshold. These modifications may allow for both energy savings during torpor as well as the rapid restoration of high mitochondrial oxidation rates during arousal. In addition, maintaining a minimal level of substrate oxidation during torpor may prevent mitochondrial membrane depolarization [and, therefore, apoptosis (Ly et al., 2003)]. Indeed, we have previously shown that mitochondrial membrane potential remains fairly constant during torpor in ground squirrels (Brown et al., 2012), even at 10°C.

The kinetics of mitochondrial respiration should reflect the underlying kinetics of those components of mitochondrial metabolism that bind succinate prior to its oxidation, in particular, the dicarboxylate transporter (DCT) and SDH. In liver, we observed that SDH had a greater apparent affinity for succinate during torpor than IBE, regardless of assay temperature. A similar trend was observed in skeletal muscle at 37°C, but it did not reach statistical significance. Therefore, increased affinity of SDH for succinate may contribute to
There was no significant interaction between metabolic state and tissue measured at 37°C (i.e. significant effect of metabolic state and tissue measured at 37°C).‡ Corresponding bar.

Mitochondrial respiration kinetics, the App

enzymes from hibernating animals generally have a greater affinity for several substrates in hibernating compared with active jerboas when measured previously for succinate kinetics of DCT [Palmieri et al., 1971]. Therefore, it seems likely that the kinetics of other metabolic components of oxidative phosphorylation at low succinate levels in this tissue, but the DCT has been shown to exhibit considerable control over succinate-fuelled respiration at low succinate levels (Quagliariello and Palmieri, 1968) and may contribute to mitochondrial suppression in this tissue in vivo. It is known that the distribution of control over mitochondrial respiration varies between tissues (Rossignol et al., 2000); therefore, it seems reasonable that the mechanisms that bring about suppression of succinate oxidation during torpor may vary between tissues accordingly.

Unlike at 37°C, SDH activity did not differ between torpor and IBE when measured at 10°C in either liver or skeletal muscle, despite mitochondrial respiration being suppressed in both tissues at this low temperature. Therefore, SDH inhibition probably only contributes to the initiation of mitochondrial metabolic suppression during entrance into torpor, when Tb is still high. Indeed, the maximal extent of active mitochondrial suppression occurs very early during entrance into torpor, before Tb falls to 30°C (Chung et al., 2011). At the same time, some mechanism other than SDH must maintain mitochondrial metabolic suppression during torpor, when Tb is low. Two previous studies (Fedotcheva et al., 1985; Armstrong and Staples, 2010) suggested that OAA binding inhibits SDH activity during torpor, but tissue OAA levels do not change between hibernation metabolic states in ground squirrel liver (Armstrong and Staples, 2010). While tissue levels do not necessarily reflect intramitochondrial OAA levels, this observation suggests that the affinity of SDH for OAA is much greater during torpor in both liver and skeletal muscle regardless of assay temperature, despite SDH not being inhibited at 10°C. This observation strengthens the contention that OAA plays a role in SDH inhibition during torpor, but also suggests that the lack of SDH inhibition at 10°C does not result from the loss of bound OAA. It may be, instead, that SDH is less temperature sensitive during torpor than IBE.

Perspectives and significance

The present study examined mitochondrial function in hibernating animals over a wide range of succinate concentrations that almost undoubtedly span the physiological succinate range. We chose to

SDH inhibition may initiate – but does not maintain – suppression of succinate-fuelled metabolism during torpor

How mitochondrial metabolism is suppressed during torpor remains to be fully elucidated, but a number of mechanisms have been proposed (Shug et al., 1971; Lerner et al., 1972; Fedotcheva et al., 1985; Brustovetsky et al., 1989; Brustovetsky et al., 1990; Barger et al., 2003; Armstrong and Staples, 2010; Brown et al., 2012). With regard to succinate-fuelled metabolism, our finding of lower SDH maximal activity (measured at 37°C) in liver during torpor is consistent with previous studies (Gehnrich and Aprille, 1988; Armstrong and Staples, 2010; Chung et al., 2011). For the first time, however, we also report lower maximal SDH activity in skeletal muscle during torpor. SDH inhibition in torpor was also evident at sub-saturating succinate concentrations. In liver, SDH activity correlated well with succinate-fuelled mitochondrial respiration rate at all concentrations where SDH inhibition and mitochondrial metabolic suppression were observed. Thus SDH inhibition probably plays an important role in the active suppression of liver mitochondrial metabolism in vivo. In skeletal muscle, however, while SDH activity accounted for up to 55% of the variation in mitochondrial respiration at ≥1 mmol l⁻¹ succinate, there was no relationship between SDH activity and mitochondrial respiration at either 0.1 or 0.5 mmol l⁻¹, despite both parameters being inhibited in torpid animals at these concentrations. This observation suggests that, at low substrate levels, SDH has little control over mitochondrial respiration in skeletal muscle. Our data do not permit us to assess how control of respiration is redistributed among the components of oxidative phosphorylation at low succinate levels in this tissue, but the DCT has been shown to exhibit considerable control over succinate-fuelled respiration at low succinate levels (Quagliariello and Palmieri, 1968) and may contribute to mitochondrial suppression in this tissue in vivo. It is known that the distribution of control over mitochondrial respiration varies between tissues (Rossignol et al., 2000); therefore, it seems reasonable that the mechanisms that bring about suppression of succinate oxidation during torpor may vary between tissues accordingly.

Unlike at 37°C, SDH activity did not differ between torpor and IBE when measured at 10°C in either liver or skeletal muscle, despite mitochondrial respiration being suppressed in both tissues at this low temperature. Therefore, SDH inhibition probably only contributes to the initiation of mitochondrial metabolic suppression during entrance into torpor, when Tb is still high. Indeed, the maximal extent of active mitochondrial suppression occurs very early during entrance into torpor, before Tb falls to 30°C (Chung et al., 2011). At the same time, some mechanism other than SDH must maintain mitochondrial metabolic suppression during torpor, when Tb is low. Two previous studies (Fedotcheva et al., 1985; Armstrong and Staples, 2010) suggested that OAA binding inhibits SDH activity during torpor, but tissue OAA levels do not change between hibernation metabolic states in ground squirrel liver (Armstrong and Staples, 2010). While tissue levels do not necessarily reflect intramitochondrial OAA levels, this observation suggests that the affinity of SDH for OAA is much greater during torpor in both liver and skeletal muscle regardless of assay temperature, despite SDH not being inhibited at 10°C. This observation strengthens the contention that OAA plays a role in SDH inhibition during torpor, but also suggests that the lack of SDH inhibition at 10°C does not result from the loss of bound OAA. It may be, instead, that SDH is less temperature sensitive during torpor than IBE.

Perspectives and significance

The present study examined mitochondrial function in hibernating animals over a wide range of succinate concentrations that almost undoubtedly span the physiological succinate range. We chose to

Fig. 5. Effect of oxaloacetate (OAA) on succinate dehydrogenase (SDH) kinetics. OAA is a mixed inhibitor when pre-incubated without succinate and, therefore, can bind to SDH when it is not already bound to succinate (inhibition constant, Kᵢ) and when it is already bound to succinate (inhibition constant, Kᵢ'). Kᵢ values did not differ between metabolic states, and so only Kᵢ values are shown for torpid (●) and interbout euthermic (○) animals. There was no significant interaction between metabolic state and temperature in either tissue. †P<0.05 versus interbout euthermia in the same tissue and at the same temperature. ‡P<0.05 versus the same metabolic state and tissue measured at 37°C (i.e. significant effect of temperature). Sample size is indicated in parentheses below the corresponding bar.
examine succinate-fuelled metabolism in the present study because (i) it is the most common substrate used in studies of mitochondrial metabolism in hibernating animals, and (ii) it allowed us to examine changes to an important segment of the electron transport chain without confounding effects from upstream processes that may be differentially regulated, for example, to shift fuel use away from carbohydrates rather than to suppress metabolism per se (Buck et al., 2002). For the most part, the results confirmed observations in previous studies made using saturating succinate concentrations (Gehnrich and Aprille, 1988; Muleme et al., 2006; Gerson et al., 2008; Armstrong and Staples, 2010; Chung et al., 2011; Brown et al., 2012), but two important facts were revealed by the present study. First, the degree of mitochondrial metabolic suppression during torpor is lower at sub-saturating compared with saturating succinate levels because torpid mitochondria are less sensitive to changes in succinate concentration than IBE mitochondria. Therefore, studies using saturating substrate levels may have overestimated the extent of mitochondrial metabolic suppression (and related energy savings) during torpor. Second, in skeletal muscle, although both succinate-fuelled respiration and SDH activity are reduced in torpor at all substrate concentrations, there was no correlation between the two parameters at succinate concentrations <1 mmol l\(^{-1}\). This suggests that SDH may have little control over respiration in skeletal muscle at physiological succinate concentrations and, therefore, may not play a significant role in suppressing mitochondrial metabolism during torpor in this tissue. This is not the conclusion that would be drawn using only saturating substrate concentrations. In addition, mitochondria in hibernating animals function over a range of temperatures, and, therefore, mitochondrial metabolism should be assessed at several representative temperatures. Many previous studies of mitochondrial metabolism in hibernating animals examined mitochondrial function at non-physiological temperatures [especially \(-25^\circ\text{C}\) (Fedotcheva et al., 1985; Brustovetsky et al., 1989; Martin et al., 1999)] or at euthermic temperatures only [37°C (e.g. Barger et al., 2003; Armstrong and Staples, 2010)], and extrapolated their results to the entire hibernation season. The present study clearly highlights the shortcoming of this approach. When measured at torpid-like temperatures, SDH activity was not reduced in torpor, and SDH inhibition probably plays little role in maintaining metabolic suppression during torpor when \(T_b\) is low. All of these nuances of mitochondrial metabolic regulation in hibernating animals would have been overlooked had we used only a single, saturating succinate concentration at a single assay temperature.

The results of the present study also highlight two important research directions. First, the mechanisms by which SDH is altered during torpor remain to be elucidated. Recently, Phillips et al. (Phillips et al., 2012) contended that mitochondrial oxidative phosphorylation complexes are acutely regulated via post-translational modifications. The phosphorylation state of SDH does not change throughout the hibernation season (D.J.C., J.C.L.B., B. Syzszka, N. P. A. Huner and J.F.S., unpublished observations), but SDH could be modulated via other post-translational modifications, especially acetylation (Cimen et al., 2010). Certainly, some proteins are well known to be post-translationally modified during hibernation, for example, phosphorylation of pyruvate dehydrogenase (Andrews et al., 1998) and glutamate dehydrogenase (Bell and Storey, 2010), as well as SUMOylation of several transcription factors (Lee et al., 2007). Second, mechanisms other than SDH inhibition are likely to influence the initiation and maintenance of mitochondrial succinate oxidation during torpor. These are probably other components of the substrate transport system (i.e. substrate transporters or other electron transport chain complexes), as previous work (Brown et al., 2012) has provided little evidence for a role for any component of the ADP phosphorylation subsystem (i.e. ATP synthase, phosphate and adenine nucleotide transporters). One site that we believe is worth further examination is the dicarboxylate transporter. In fact, we generally believe that more emphasis should be placed on the potential role of substrate transporters in regulating mitochondrial metabolism during torpor in hibernating mammals.

LIST OF SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>App(_{\text{Km}})</td>
<td>apparent Michaelis–Menten constant</td>
</tr>
<tr>
<td>DCT</td>
<td>dicarboxylate transporter</td>
</tr>
<tr>
<td>GDH</td>
<td>glutamate dehydrogenase</td>
</tr>
<tr>
<td>IBE</td>
<td>interbout euthermia</td>
</tr>
<tr>
<td>(K_i)</td>
<td>inhibition constant</td>
</tr>
<tr>
<td>MR</td>
<td>metabolic rate</td>
</tr>
<tr>
<td>OAA</td>
<td>oxaloacetate</td>
</tr>
<tr>
<td>SDH</td>
<td>succinate dehydrogenase</td>
</tr>
<tr>
<td>(T_b)</td>
<td>body temperature</td>
</tr>
<tr>
<td>(V_{\text{max}})</td>
<td>maximal enzyme activity</td>
</tr>
</tbody>
</table>

ACKNOWLEDGEMENTS

We thank Lynda McCaig for her assistance with animal dissection. We also thank Alvin Iverson and the staff of the Carman Area Research Center (University of Manitoba) for assistance trapping animals, and Manitoba Conservation for permission to trap animals. Thoughtful comments from two anonymous reviewers helped to improve this manuscript.

AUTHOR CONTRIBUTIONS

J.C.L.B. conceived the study and its design, conducted all respiration and enzyme activity measures, conducted data analysis, and wrote and edited the manuscript; A.N.C. isolated liver mitochondria; D.J.C. isolated skeletal muscle mitochondria; J.F.S. contributed to study design and edited the manuscript.

COMPETING INTERESTS

No competing interests declared.

FUNDING

This study was supported financially by a Discovery Grant from the Natural Sciences and Engineering Research Council (to J.F.S.), and a Helen Battle Postdoctoral Fellowship (to J.C.L.B.).

REFERENCES


Mitochondrial regulation in hibernation


